INFORMATION SHEET ON THE DETERMINATION OF ASCORBIC ACID IN FRESH, FROZEN, AND DEHYDRATED FOODS

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The method of determining ascorbic acid in foods that is described below has been discussed in detail by Loeffler and Ponting in the Analytical Edition of Industrial and Engineering Chemistry, 14:846-849, 1942. A summary of the steps involved in the method is reported below.

Blending Sample

Blend 25 or 50 grams of fresh or frozen fruit or vegetable tissue with 350 ml. of 1 percent metaphosphoric acid in a blending machine operated for five minutes at high speed. If the material is of high ascorbic acid content, such as leafy vegetables, raspberries, strawberries, or asparagus, use the smaller quantity. Fifty grams are used for fresh or frozen foods containing less ascorbic acid, such as potatoes, carrots, sweetpotatoes, peaches, plums, and apricots. If a very large sample is desired, in order to assure greater uniformity, these weights can be doubled and 650 ml. of acid extractant used. This is about the maximum capacity of the blending cups. Frozen material need not be defrosted prior to blending. When a dehydrated fruit or vegetable is being analyzed, 5 or 10 grams of sample and 350 ml. of extractant are used, in accordance with the classification mentioned above. If metaphosphoric acid is not available, the same volume of 0.25-percent oxalic acid may be substituted as the extractant (J. D. Ponting, Indus. and Engin. Chem., Anal. Ed., 15:389-391, 1943).

Clarification of Extract

After blending the extracts should be clarified, preferably by centrifugation. Moderate turbidities do not interfere, since the instrument is calibrated with proper blanks. Extracts of starchy vegetables that filter slowly (such as the extract of potato) may lose considerable ascorbic acid during filtration but will lose none during centrifugation. Even with centrifugation the extracts should be tested within a few minutes to avoid loss of ascorbic acid.

Testing and Calculations

Pipette 1-ml. portions of the centrifugate into three matched tubes from the Evelyn photoelectric colorimeter. Add 9 ml. of distilled water to one tube and adjust the colorimeter to read 100 with this tube, using filter No. 520. To each of the other tubes add 9 ml. of the previously standardized indophenol dye solution from a calibrated rapid-delivery pipette. (Standardization of dye is discussed below.) Take a reading in the photoelectric colorimeter, using filter No. 520, ten seconds after the beginning of the addition of dye.

This reading is G_2, from which the corresponding L_2 value is obtained from the calibration chart provided with the instrument. This value (L_2) and the value for L_1 (obtained in the dye standardization) are substituted in the following equation, which includes the factor 10.8, determined by Evelyn, Malloy, and Rosen (Jour. Biol. Chem., 126:645-654, 1938).

$$\text{Ascorbic acid (mg. per 100 gm.)} =$$

$$10.8(L_1-L_2)\ \frac{\text{ml. acid extractant} + (\text{pct. liquid in sample})(\text{gm. sample})}{\text{gm. sample}}$$

For most fresh or frozen fruits the sum of the percentages of soluble solids and of water will be so near 100 that the insoluble material can be neglected. The formula then becomes:

$$\text{Ascorbic acid (mg. per 100 gm.)} = 10.8(L_1-L_2)\frac{\text{ml. acid} + \text{gm. sample}}{\text{gm. sample}}$$

For dehydrated vegetables the formula becomes:

$$\text{Ascorbic acid (mg. per 100 gm.)} = 10.8\,(L_1-L_2)\ \frac{(\text{ml. acid})}{(\text{gm. sample})}$$

Standardization of Dye

The dye is standardized by noting the 10-second reading with filter No. 520 (when the instrument is calibrated to 100 with distilled water) given by a tube containing 1 ml. of 1 percent metaphosphoric acid (or 0.25-percent oxalic acid when used) and 9 ml. of the dye solution. This value is G_1, from which L_1 is obtained by reference to the calibration chart. L_1 is then substitutdd in the equation above.

The dye solution is prepared simply by dissolving enough of the dye in water so that a G_1 reading of about 30 is given with the Evelyn photoelectric colorimeter. The concentration of dye to give such a reading is roughly 13 mg. per liter.

Suggestions and precautions

A reservoir-type automatic pipette for the acid and nonreservoir types for the 1-ml. portions of filtrate and 9-ml. portions of dye will be found advantageous. The 9-ml. pipette must extend to near the surface of the liquid in the tube to avoid splashing and must be calibrated to drain uniformly in less than 5 seconds.

For material low in ascorbic acid such as potatoes and carrots, the ratio of extract to dye can be altered to give greater sensitivity. The use of 2 ml. of centrifugate plus 9 ml. of dye is also satisfactory, since the sensitivity is doubled. The factor 6.25 must then be used instead of the 10.8 given in the formulas above. A larger proportion of extract to dye is not recommended, since the fading of the dye will be accelerated.

For most fruits and vegetables the fading of the dye when added to the extracts in a 9-plus-1 ratio will be no greater than the fading of the dye in the acid alone--that is, not over one-fourth scale division in a 10-second interval. Where the tissue extract contains interfering substances which cause greater fading, an approximate correction should be made. This can be accomplished by taking both a 10- and a 20-second reading and subtracting the difference between the two.

Violent splashing and loss of liquid may occur when the blenders are started. Bits of dehydrated material will be thrown out of the liquid and may stick on the upper surfaces of the container, where they do not become macerated and extracted. These losses can be prevented by using auto-transformers in series with the blenders so that they serve as starting switches. The starting impulse can then be applied gradually.

It is difficult to calibrate the automatic 1-ml. pipette to deliver exactly 1 ml. It is simpler to adjust the pipette to approximately 1 ml. and then determine the exact factor for each pipette by testing a solution of pure ascorbic acid of known concentration, freshly prepared in acid. This factor is then used instead of the 10.8 mentioned previously.

Although the factor 10.8 given by Evelyn has been confirmed many times, one worker has reported that he obtained a factor other than 10.8 by using the suggested amount of ascorbic acid solution and dye, the Evelyn colorimeter, and the proper filter. In case of doubt, therefore, the instrument itself can be checked by determining whether the proper factor is obtained on a pure, freshly prepared solution of ascorbic acid.

Photoelectric colorimeters other than the Evelyn can be used, provided the cells permit very rapid mixing and the instrument can be read rapidly. The factor must be redetermined for the specific cells, filter, and instrument used.